REMARKS

A check for the requisite fee for a one-month extension of time is enclosed. Any fees that may be due in connection with the filing of this paper or with this application should be charged to Deposit Account No. 02-1818. If a Petition for Extension of Time is needed, this paper is to be considered such Petition. A Supplemental Information Disclosure Statement accompanies this response.

Claims 1-11, 13-17, 19-29 and 31-37 are pending. Claims 19-29 and 31-37, which are directed to non-elected subject matter, are withdrawn from consideration but are retained for possible joinder upon allowance of a linking claim. Claim 1 is amended for clarity. Basis for the amendment is found, *e.g.*, at page 4, lines 17-20. No new matter is added.

RESTRICTION REQUIREMENT

The Requirement for restriction as modified in the Decision on Petition, mailed August 26, 2008. rejoining Groups I and II, is acknowledged. Accordingly, claims 1-11 and 13-17, drawn to a bi-phasic culture medium and kits containing the culture medium, which have been examined on the merits, are elected.

REJECTION OF CLAIMS 1-11 AND 13-17 UNDER 35 U.S.C. § 103(a)

Claims 1-11 and 13-17 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Clark *et al.* (Clin. Microbiol. Rev. 15(3): 329-341 (2002)) in view of Nakamura (Bacteriol Rev 17(3): 189-212 (1953)) and Shimakita *et al.* (US 2003/0003527 Al) and Petri *et al.* (US 5,272,058) because Clark *et al.* teaches a bi-phasic culture medium that allegedly includes every element of the instantly claimed medium except peptone and an antibiotic, and does not teach kits, but Nakamura allegedly teaches media that include peptones and antibiotics, such as penicillin, and Shimakita *et al.* and Petri *et al.* teach kits. The Examiner alleges that it would have been obvious to one of ordinary skill in the art to modify the media described in Clark *et al.* to add peptones and antibiotics, based upon the teachings of Nakamura with respect to the art-recognized benefits of adding peptones and antibiotics to culture media, and to include such media in kits as taught by Shimakita *et al.* and Petri *et al.* The Examiner alleges that the result-effective adjustment of particular concentrations of ingredients within the medium, providing particular ingredients, such as particular antibiotics or peptones, is merely a matter of judicious selection and routine optimization which is well within the purview of the skilled artisan. This rejection respectfully is traversed.

Relevant Law

The relevant law with respect to obviousness is discussed in the previous response, and is incorporated herein by reference.

The phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention. *In re Herz*, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976) (emphasis in original). For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, ***absent a clear indication in the specification or claims of what the basic and novel characteristics actually are***, "consisting essentially of" will be construed as equivalent to "comprising." See, *e.g.*, *PPG Industries v. Guardian Industries,* 156 F.3d 1351, 1354, 48 USPQ2d 1351, 1353-54 (Fed. Cir. 1998), [emphasis added].

In this instance, as discussed below, the application provides a clear indication of the basic and novel characteristics. Thus, "consisting essentially of" should **not** be construed as "comprising."

The Claims

Claim 1 recites a bi-phasic culture medium that includes a solid phase containing an egg slope or agar slope and a live *Escherichia coli*-free liquid phase consisting essentially of: serum, a peptone, a phosphate buffered saline and optionally an antibiotic. Claims 2-11 ultimately depend from claim 1 and include every limitation thereof. Claims 13-17 recite kits that include the medium of claim 1. Thus, claim 1 is directed to a simplified bi-phasic medium, where the liquid phase is free of live *Escherichia coli* and contains serum, a peptone, a phosphate buffered saline and optionally an antibiotic and only other ingredients that do not materially affect basic and novel characteristics of the liquid phase.

Analysis

Before addressing the Examiner's reasoning in detail, it does not appear that the Examiner appreciates that the instant application describes a new simplified culture medium that is a less complex medium for growing protozoa, such as *Dientamoeba fragilis*, than those described in the art. Bi-phasic media described in the prior art include a liquid phase containing live bacteria, such as *E. coli*, and numerous chemicals and require special mixing protocols or processing. The instantly claimed bi-phasic medium has a liquid phase that does not include live *E. coli* bacteria, includes fewer ingredients than the prior art media, and by culture standards is greatly simplified. Because the instantly claimed medium does not include live *E. coli*, has fewer ingredients and does not have the special processing or handling restrictions of the prior art media, it is simplified, and, as taught in the application, very reliable. The claims capture the fact that the medium is simplified by reciting that the liquid phase is live *Escherichia coli*-free and "consists essentially of" the recited ingredients.

The phrase "consisting essentially of" can be construed as equivalent to "comprising" for the purposes of searching for and applying prior art under 35 U.S.C. § 102 and §103 if there is no clear indication in the specification or claims of the basic and novel characteristics of the claimed subject matter. Applicant respectfully submits that, in this instance, the specification provides a clear indication of basic and novel characteristics of the claimed subject matter. The instant specification, *e.g.*, at page 4, lines 4-20, describes the instant medium as a simplified medium that a) is less complex because it requires fewer ingredients but still supports the growth of protozoa, b) can be used as a transport medium, c) demonstrates enhanced reliability and d) eliminates the need for a bacterial flora such as *E. coli*. The specification states that the instant medium is greatly simplified yet more reliable than prior art media (*Id.*). Thus, compared to the prior art media, the instant medium is easier to prepare and use, is more reliable and is easily transportable. Hence, the specification provides a clear indication of basic and novel characteristics of the claimed subject matter that distinguish it from the prior art.

Because the specification provides a clear indication of basic and novel characteristics of the claimed subject matter, the phrase "consisting essentially of" must be construed as limiting the scope of the limitation in the claim to the recited materials "and those that do not materially affect the basic and novel characteristic(s)" of the claimed medium. *In re Herz*, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976) (emphasis in original). Thus, the cited art must teach or suggest (1) simplifying a bi-phasic media, including elimination of bacteria, (2) the recited ingredients in the liquid phase of the instantly claimed medium and (3) the results achieved thereby. The cited art, alone or in any combination, does not do so.

The teachings of the cited art and differences from the claimed subject matter.

Clark *et al.*

Clark *et al.* teaches that it is not possible to grow some amoeba, such as *D. fragilis*, in an axenic culture (medium that does not contain a live bacterial flora; see page 330). Clark *et al.* describes bi-phasic Robinson's medium and bi-phasic LE medium for xenic cultures (see pages 332-333). Bi-phasic Robinson's medium is a complex medium that includes live *E. coli* and other ingredients in the liquid phase. The preparation of Robinson's medium involves the preparation of six separate aqueous stock solutions: (a) 0.5% erythromycin; (b) 20% bactopeptone; (c) potassium hydrogen phthalate and sodium hydroxide; (d) "R medium," which contains sodium chloride, citric acid, potassium phosphate monobasic, ammonium sulfate, magnesium sulfate and lactic acid; (e) "BR medium," which is "R medium" as

described in (d) inoculated with a live standard *E. coli* strain; and (f) "BRS medium," which is "BR medium" as described in (e) to which an equal volume of heat inactivated bovine serum is added (see page 333). Aliquots of solutions (a), (b), (c) and (f) are combined to form a liquid phase, which is layered over an agar slant to produce a bi-phasic medium. Thus, the Robinson's medium described in Clark *et al.* includes live *E. coli* and ingredients other than recited in the liquid phase as instantly claimed. The Robinson's medium is a complex medium requiring the preparation of six different stock solutions and separate sub-combinations of the solutions.

Clark *et al.* also describes bi-phasic LE medium. The bi-phasic LE medium includes a liquid phase containing live bacteria and Locke's solution (an aqueous solution of sodium chloride, calcium chloride, potassium chloride, magnesium chloride, monobasic and dibasic sodium phosphate, sodium bicarbonate and monobasic potassium phosphate) overlayering an egg slant (page 332).

Clark *et al.* teaches that the ingredients of the liquid phase must be combined in a certain order. Clark *et al.* teaches that, when preparing the LE medium, it may be necessary after sterilization to filter the medium to remove any precipitates followed by re-sterilization. Thus, the LE medium described in Clark *et al.* is complex, containing numerous ingredients and having special processing requirements. The liquid phase of the LE medium described in Clark *et al.* includes live bacteria. The liquid phase also contains ingredients other than a serum, a peptone, a phosphate buffered saline and an antibiotic, and these additional ingredients would affect the basic and novel characteristics of the liquid phase as instantly claimed. For example, the LE medium described in Clark *et al.* contains bacteria and other components, including calcium chloride, magnesium chloride and sodium bicarbonate. The presence of calcium chloride and/or magnesium chloride and/or sodium bicarbonate can materially affect the basic and novel characteristics of the liquid phase because bicarbonate and phosphate tend to precipitate in the presence of magnesium and calcium ions (see U.S. Pat. No. 4,975,410, col. 1, lines 58-60), thereby eliminating the ease of preparation and its stability during storage. It is known in the art that use of bicarbonate in solutions that must be sterilized and stored for a prolonged period of time results in problems since bicarbonate is metastable and is transformed into carbonate under emission of carbon dioxide, and that the inclusion of calcium and magnesium ions in such solutions can result in calcium carbonate precipitation (*e.g.*, see Becker, WO00/57833, page 1, lines 31-37, previously provided). Clark *et al.* states that salts in the aqueous phase of its LE medium may precipitate after sterilization,

necessitating additional filtration steps to remove any precipitate and further sterilization (see page 332). This precipitation of salts also can result in batch-to-batch variation of the liquid phase due to the variation in any precipitation and subsequent filtration. Thus, adding the salts of Clark *et al.* to the liquid phase of the medium as instantly claimed would affect the basic and novel characteristics of the instantly claimed liquid phase because it would make the claimed medium more complex, more difficult to prepare, less stable on storage and less reliable because of possible variation between batches.

Significantly, there is no teaching or suggestion in Clark *et al.* for simplifying the liquid phase of its bi-phasic media, such as by eliminating live *E. coli* or any of the ingredients in the liquid phase of the media described therein. Therefore, Clark *et al.* fails to teach or suggest (1) simplifying the liquid phase of its media, including eliminating the live *E. coli* bacteria, (2) a bi-phasic medium that has a live *Escherichia coli*-free liquid phase containing serum, a peptone, a phosphate buffered saline and optionally an antibiotic and only other ingredients that do not materially affect the basic and novel characteristics of the liquid phase and (3) the results achieved thereby. The secondary references fail to teach or suggest these elements.

Nakamura

Nakamura does not cure these deficiencies. Nakamura teaches the nutrition requirements of the amoeba *Entamoeba histolytica*, including its growth in xenic culture using bi-phasic Locke-egg-serum (LES) medium containing bacteria. Nakamura teaches that, except for some reports of temporarily maintained cultures, *E. histolytica* has not been cultivated free of an associated organism and that bacteria are absolutely essential for the cultivation of amoeba (page 201, first full paragraph). Nakamura teaches that the amount of nutritional material present in killed bacterial cells is inadequate to propagate amoeba (page 192).

Nakamura teaches that various simplifications of the LES medium have been proposed in the art, including eliminating the egg slant to provide a liquid medium containing only Locke's solution and serum or normal saline and inactivated serum, or a medium containing an infusion of coagulated egg yolk in buffered salt solution, or a medium containing sterile nutrient broth and a mixture of starch and charcoal or a medium containing extracted powdered heart muscle in a modified Locke's solution. These media are not bi-phasic and Nakamura suggests that these modified monophasic media are not suitable for amoeba propagation (page 190). Nakamura teaches that most of the modifications of the original bi-phasic medium have been changes in the solid portion of the medium (page 191). Thus, Nakamura does not suggest simplifying the liquid phase of a bi-phasic medium.

Nakamura does not teach the elimination of live *E. coli* from its media. Nakamura teaches that all the media used to grow amoeba contain bacteria (page 192, third paragraph). Nakamura teaches of a publication in the art that suggests that LE medium conditioned by the cultivation of *E. coli* in it for 24 hours to provide an *E. coli* growth factor, followed by heat inactivation of the *E. coli*, supported amoeba growth, but that the publication provided no proof that live *E. coli* were completely absent (page 192). Nakamura teaches that the complexity of the growth requirements is such that it is difficult to establish any single growth factor for amoeba such as *E. histolytica*, but that blood, serum, egg, liver, liver extract, peptone and rice powder have been used for cultivating amoeba and that these materials apparently include some growth factor or growth factor stimulant for amoeba (page 195). Thus, Nakamura describes several alternative media for growing amoeba, but does not teach or suggest simplifying the liquid phase of a bi-phasic medium to eliminate live *E. coli*, or to contain serum, a peptone, a phosphate buffered saline and optionally an antibiotic and only any other ingredients that do not materially affect the basic and novel characteristics of the liquid phase.

The only mention of peptone in Nakamura is that blood, serum, egg, liver, liver extract, peptone and rice powder have been used for the cultivation of is *E. histolytica*, and that these materials may include some growth factor or growth factor stimulant for *E. histolytica*. There is no teaching or suggestion in Nakamura that the combination of serum, peptone and phosphate buffered saline and optionally an antibiotic can replace the live bacterial flora, such as live *E. coli*, and calcium chloride, magnesium chloride and sodium bicarbonate of Locke's solution of the LE medium described in Clark *et al.* Therefore, Nakamura fails to teach or suggest (1) simplifying the liquid phase of its bi-phasic media, including elimination of live *E. coli* bacteria, (2) a bi-phasic medium that has a live *Escherichia coli*-free liquid phase containing serum, a peptone, a phosphate buffered saline and optionally an antibiotic and only other ingredients that do not materially affect the basic and novel characteristics of the liquid phase and (3) the results achieved thereby.

Shimakita *et al.* (US 20030003527 Al)

Shimakita *et al.* does not cure the deficiencies in the teachings of Clark *et al.* and Nakamura. Shimakita *et al.* teaches kits for detecting microorganisms. The kits include compounds for staining living and dead cells. In some embodiments, the microorganism detecting kit contains a culture medium for culturing microorganisms. Shimakita *et al.* describes the culture medium as a solid that contains peptone, sodium chloride, agar and 4',6-diamidino-2-phenylindole dihydrochloride for staining living and dead cells. Shimakita *et al.*

also teaches that a commercially available normal agar culture medium (made by Nissui Seiyaku, Co.) can be used as the culture medium but provides no description of this medium.

Shimakita *et al.* does no teach or suggest a bi-phasic medium. Shimakita *et al.* does not teach or suggest simplifying any media. Therefore, Shimakita *et al.* fails to teach or suggest the elements missing from the combined teachings of Clark *et al.* and Nakamura.

Petri *et al.* (US 5,272,058)

Petri *et al.* does not cure the deficiencies in the combined teachings of Clark *et al.*, Nakamura and Shimakita *et al.* Petri *et al.* describes growing a pathogenic strain of *E. histolytica* in liquid medium TYIS-33 (trypticase yeast extract, iron and serum) with 100 U/ml penicillin and 100 mg/ml streptomycin sulfate. Petri *et al.* also describes growing pathogenic and nonpathogenic strains in TYSGM-9 medium containing rice starch in the presence of bacterial flora and growing nonpathogenic amoeba in Robinson's medium or TYIS-33 containing bacteria. The only bi-phasic medium mentioned in Petri *et al.* is Robinson's medium (TYSGM-9 is monophasic; see Clark *et al.*). The composition of Robinson's medium is described in Clark *et al.* and discussed above. The liquid phase of Robinson's medium includes live *E. coli* bacteria. There is no teaching or suggestion in Petri *et al.* to modify Robinson's medium nor to simplify the liquid phase of Robinson's medium nor to eliminate the live *E. coli* bacteria or any of the ingredients in the liquid phase of Robinson's medium to arrive at the medium as instantly claimed. Therefore, Petri *et al.* fails to teach or suggest (1) simplifying the liquid phase of its bi-phasic media, including eliminating live *E. coli* bacteria, (2) a bi-phasic medium that has a live *Escherichia coli*-free liquid phase containing serum, a peptone, a phosphate buffered saline and optionally an antibiotic and only other ingredients that do not materially affect the basic and novel characteristics of the liquid phase and (3) the results achieved thereby.

Analysis

The Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* does not result in the instantly claimed culture medium nor kits

Applicant respectfully submits that the combination of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* does not result in the instant claims. The claims recite a bi-phasic culture medium that includes a solid phase containing an egg slope or agar slope; and a live *Escherichia coli*-free liquid phase containing only serum, a peptone, a phosphate buffered saline and optionally an antibiotic as ingredients that materially affect the basic and novel

characteristics of the liquid phase, and kits that include the medium. The specification recites that the liquid phase of the claimed bi-phasic medium has been simplified in comparison to media described in the art. For example, see page 4, lines 4-20, which recites:

> In the present invention the culture method and medium has been simplified to a less complex medium but one that will in use, support the growth of the protozoa referred above including *D fragilis*, *Blastocystis hominis* (*B. hominis*) and other parasites including other amoebae (for example *E. histolytica/dispar*, *Iodamoeba buetschlii, Endolimax nana, Entamoeba coli, Entamoeba hartinanni* being other pathogens). The medium in accordance with the invention can double as a transport medium where a sample is taken off-site from the laboratory and then transported to the laboratory. The culture medium preferably uses an egg slope as opposed to previous used saline agar cultures. The egg slope may be made by any appropriate method known in the art, for example by diluting hen's eggs 50/50 in either Ringer's salt solution or PBS (phosphate buffered saline). The culture medium of the invention demonstrates enhanced reliability of culture. The medium is not as complex as those described in the prior art which comprise numerous chemicals. The medium in accordance with the present invention is--by culture standards--greatly simplified yet more reliable. Unlike the use of live *E. coli* bacteria (which are not suitable to be given to patients in order to collect their own specimens), the medium in accordance with the invention is designed to work even more reliably without E. coli.

The specification describes the claimed liquid phase as less complex because it requires fewer ingredients, is easier to prepare and use, is more reliable, eliminates the need to include live *E. coli* bacteria, has a long shelf life and is easily transportable. Hence, the specification provides a clear indication of the basic and novel characteristics of the claimed medium.

Simplifying the liquid phase

The combined teachings of Clark *et al.*, Nakamura, Shimakita *et al.* and Petri *et al.* does not teach or suggest simplifying the liquid phase of its bi-phasic media, including eliminating live *E. coli* bacteria. Clark *et al.* teaches that bacteria are required for growing certain amoeba, such as *D. fragilis*, and that Robinson's medium includes live *E. coli* bacteria. Nakamura teaches that bacteria are essential for the cultivation of amoeba. Nakamura does not teach or suggest eliminating live *E. coli* from LE medium or Robinson's medium. Shimakita *et al.* is silent with respect to a bi-phasic medium or a bacterial flora, and Petri *et al.* teaches growing amoeba, such as *E. histolytica*, in bi-phasic Robinson's medium containing live *E. coli* bacteria in the liquid phase. None of Clark *et al.*, Nakamura, Shimakita *et al.* nor Petri *et al.*, alone or in any combination, teaches or suggests simplifying the liquid phase of the bi-phasic LE medium or Robinson's medium to eliminate live *E. coli* bacteria, nor does the combination of the cited references suggest that such modification would yield a culture media that would support the growth of amoeba. The combination of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* does not teach or suggest eliminating calcium chloride and/or magnesium chloride and/or magnesium sulfate and/or sodium bicarbonate from the liquid phase, nor

provide any rationale for doing so. Thus, the combined teachings of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* fails to teach or suggest eliminating live *E. coli* or any ingredient from the liquid phase of a bi-phasic medium to produce a simplified liquid phase. For these reasons, the combined teachings fail to result in the instant claims.

Consisting essentially of serum, a peptone, a PBS and optionally an antibiotic

In addition, the combined teachings of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* fail to teach or suggest a bi-phasic medium that has a live *Escherichia coli*-free liquid phase containing serum, a peptone, a phosphate buffered saline (PBS) and optionally an antibiotic and only other ingredient(s) that do not materially affect the basic and novel characteristics of the liquid phase.

Shimakita *et al.* does not describe a bi-phasic medium and the liquid phase of the bi-phasic media described in Clark *et al.* and Nakamura and Petri *et al.* contains other ingredients that would affect the basic and novel characteristics of the liquid phase as instantly claimed. For example, the media described in Clark *et al.* and Nakamura and Petri *et al.* include calcium chloride and/or magnesium chloride and/or magnesium sulfate and/or sodium bicarbonate. The presence of calcium ions and/or magnesium ions and/or sodium bicarbonate can materially affect the basic and novel characteristics of the liquid phase because the presence of such salts increases the number of ingredients in the medium and also can eliminate the ease of preparation of the medium. In addition, Clark *et al.* states that its combination of salts may precipitate after sterilization, necessitating additional filtration steps to remove any precipitate and further sterilization (see page 332). It is well known in the art that bicarbonate and phosphate tend to precipitate in the presence of magnesium and calcium ions (see U.S. Pat. No. 4,975,410, col. 1, lines 58-60), thereby eliminating the ease of preparation and the stability of the medium during storage. This precipitation of salts also can result in batch-to-batch variation of the liquid phase due to the variation in any precipitation and subsequent filtration. This precipitation of salts also can result in reduced shelf life due to precipitation with age. Thus, including the salts present in the media described in Clark *et al.* in the liquid phase of the medium as instantly claimed would affect the basic and novel characteristics of the instantly claimed liquid phase because it makes the liquid phase of the medium more complex, more difficult to prepare, less reliable because of possible variation between batches and less stable due to precipitation with age. Therefore, the magnesium and/or calcium and/or bicarbonate salts present in the media of Clark *et al.*, Nakamura and/or Petri *et al.* are excluded from the liquid phase of the medium as instantly claimed.

Conclusion

There is no teaching or suggestion in the combined teachings of Clark *et al.*, Nakamura, Shimakita *et al.* and Petri *et al.* to simplify a bi-phasic medium by eliminating live *E. coli* bacteria and reducing the number of ingredients in the liquid phase to only serum, a peptone, a phosphate buffered saline and optionally an antibiotic and only those other ingredients that would not materially affect the basic and novel characteristics of the liquid phase. Therefore, the combination of teachings of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* does not result in the instantly claimed medium nor kits. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness of any pending claim.

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In view of the amendment and remarks herein, reconsideration and allowance respectfully are requested.

Respectfully submitted,

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